Perspective: L-arginine and L-citrulline Supplementation in Pregnancy: A Potential Strategy to Improve Birth Outcomes in Low-Resource Settings

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ABSTRACT

The available data support the hypothesis that L-arginine or L-citrulline supplementation would be suitable for implementation in resource-constrained settings and will enhance placental vascular development and improve birth outcomes. In resource-constrained settings, the rates of adverse birth outcomes, including fetal growth restriction, preterm birth, and low birth weight, are disproportionately high. Complications resulting from preterm birth are now the leading cause of mortality in children <5 y of age worldwide. Despite the global health burden of adverse birth outcomes, few effective interventions are currently available and new strategies are urgently needed, especially for low-resource settings. L-arginine is a nutritionally essential amino acid in pregnancy and an immediate precursor of nitric oxide. During pregnancy, placental and embryonic growth increases the demand for L-arginine, which can exceed endogenous synthesis of L-arginine from L-citrulline, necessitating increased dietary intake. In many low-resource settings, dietary intake of L-arginine in pregnancy is inadequate owing to widespread protein malnutrition and depletion of endogenous L-arginine due to maternal infections, in particular malaria. Here we examine the role of the L-arginine–nitric oxide biosynthetic pathway in pregnancy including placental vascular development and fetal growth. We review the evidence for the relations between altered L-arginine bioavailability and pregnancy outcomes, and strategies for arginine supplementation in pregnancy. Existing studies of L-arginine supplementation in pregnancy in high-resource settings have shown improved maternal and fetal hemodynamics, prevention of pre-eclampsia, and improved birth outcomes including higher birth weight and longer gestation. Arginine supplementation studies now need to be extended to pregnant women in low-resource settings, especially those at risk of malaria. *Adv Nutr* 2019;10:765–777.

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Adverse Birth Outcomes Are Disproportionately High in Low-Resource Settings

Complications resulting from preterm birth (PTB; delivery before 37 weeks of gestation) are the leading cause of death in children under the age of 5 y (1, 2). An estimated 15 million preterm deliveries occur each year and 1 million preterm infants die in their first month of life owing to complications of prematurity including birth asphyxia, hypothermia, infection, and respiratory distress syndrome (3). A further 800,000 infant deaths every year are attributed to fetal growth restriction (FGR) (4). Among newborns that survive, PTB and FGR are associated with poor long-term development including childhood stunting, neurological impairments, and an increased risk of chronic disease (4–6). Despite the growing global burden of PTB and other adverse birth outcomes, there are a limited number of safe and effective intervention strategies to improve health outcomes in pregnancy.

The likelihood of delivering preterm is greatly increased in resource-constrained settings. More than 60% of all PTBs and three-quarters of neonatal deaths resulting from PTB complications occur in South Asia and sub-Saharan Africa (1). The disparity in adverse birth outcomes between high-income countries and low- and middle-income countries (LMICs) is due in large part to modifiable risk factors including infectious disease (e.g., malaria, HIV, and sexually

transmitted diseases), anemia, undiagnosed pre-eclampsia, and maternal malnutrition (7).

Maternal Malnutrition and Infection Are Key Contributors to Adverse Birth Outcomes in Resource-Constrained Settings

Malnutrition is a key contributor to global maternal morbidity and mortality, adverse birth outcomes, and long-term health consequences for the child (8–10). In 2016, an estimated 815 million individuals worldwide were chronically malnourished, with the vast majority living in sub-Saharan Africa and South Asia (11). Protein deficiency and poor-quality protein consumption (e.g., cereals lacking essential amino acids) are widespread across sub-Saharan Africa (12, 13) and low circulating concentrations of essential and conditionally essential amino acids including L-arginine are associated with impaired child growth (13). Infants, children, and women of reproductive age are at particular risk of nutritional deficiencies given their increased requirements (8). Over 10% of reproductive-aged women in LMICs are underweight (BMI <18.5 kg/m^2) (4, 14, 15). Poor nutritional status, reflected by low BMI, insufficient weight gain across pregnancy, and micro- and macronutrient deficiencies, contributes to increased risk of PTB, FGR, and neurodevelopmental deficits in children (8, 9, 16, 17). Protein supplementation has been shown to reduce rates of adverse pregnancy outcomes. Systematic reviews of intervention studies using balanced-energy protein in pregnancy have reported significant reductions in the incidence of small-for-gestational-age outcomes and stillbirth (18, 19). However, high rates of adverse birth outcomes still occur and more targeted strategies (e.g., L-arginine supplementation) may be required to further reduce poor birth outcomes, especially those related to common infections in pregnancy (e.g., malaria and HIV).

In this Perspective, we examine malaria in pregnancy as a model of infections whose negative impact on birth outcomes could be mitigated by arginine supplementation. Worldwide, 125 million pregnancies are at risk of malaria infection each year (20). Malaria infections are more frequent and severe during pregnancy and result in an estimated

750,000 low-birth-weight deliveries (due to PTB and/or FGR) in sub-Saharan Africa alone (21, 22). Considerable evidence supports an interaction between malaria in pregnancy and maternal malnutrition in worsening pregnancy outcomes in malaria-endemic regions (23). A recent meta-analysis reported an increased risk of low birth weight in women who were both malnourished and exposed to malaria, compared with women affected by either risk factor alone (24). Identification of common pathways leading to adverse birth outcomes affected by both malaria infection and malnutrition would enable more targeted and effective prevention strategies. Here we examine the role of one such common pathway, the L-arginine–nitric oxide (NO) biosynthetic pathway. The L-arginine–NO pathway is important for healthy pregnancies, dependent on maternal nutritional status, and negatively affected by maternal infections including malaria. We hypothesize that a combination L-arginine and L-citrulline supplement for pregnant women in LMICs at risk of malaria infection could increase bioavailable NO, enhance placental vascular development, and improve birth outcomes.

L-arginine–NO Biogenesis Plays a Critical Role in Placental Vascular Development

The formation of a functional and adequately vascularized placenta is essential for a healthy pregnancy and birth outcome (25). The establishment of vascularized placental tertiary villi, the site of nutrient exchange between maternal and fetal blood, depends on the tightly regulated processes of vasculogenesis, or the de novo formation of blood vessels, and angiogenesis, the remodeling of existing vasculature (26, 27).

A growing body of evidence links placental vascular pathology with poor fetal growth and adverse birth outcomes (28–30). Altered uterine (maternal side) and umbilical (fetal side) hemodynamics resulting from disruptions to normal placental vasculogenesis or angiogenesis has been mechanistically linked to adverse outcomes including fetal hypoxia, hypoglycemia, and impaired fetal organ growth (31, 32). Moreover, insufficient vascular adaptation to accommodate changing hemodynamic activity as the fetus grows can contribute to maternal gestational diabetes, pre-eclampsia, and adverse birth outcomes such as PTB and FGR (33, 34).

L-arginine is involved in multiple pathways that contribute to healthy placental and fetal function and development. L-arginine is the physiologically active isomer of arginine (compared with the D-isomer) and thus the supplementation substrate; therefore, we refer to L-arginine as "arginine" in this article. The body can produce arginine via de novo synthesis from L-citrulline, as well as during protein turnover; therefore, exogenous arginine is not the sole source. De novo synthesis of arginine accounts for 5–15% of circulating plasma arginine (35). Endogenous arginine synthesis involves a diverse set of enzymes that are differentially expressed across organs and cell types. Adult mammals synthesize most de novo arginine in the kidney; however, the location of synthesis and its relative

contribution to circulating arginine depend on species and stage of development (35). Net production of arginine in a given organ is determined by the relative expression of arginine synthesis and arginine catabolic enzymes (35).

Arginine is a precursor in numerous biological pathways including protein synthesis and the production of ornithine and urea, glutamate, NO, creatine, proline, and polyamines (35, 36). The complex metabolic pathways of arginine have been well characterized (35). A number of enzymes and competitive inhibitors including arginase and asymmetric dimethylarginine (ADMA) influence the equilibrium of arginine use between metabolic pathways, dictating the bioavailability of NO (**Figure 1**). Three principal isoforms of nitric oxide synthase (NOS) enzymes produce NO from arginine: neuronal NOS (nNOS or NOS1) and endothelial NOS (eNOS or NOS3) which are both constitutively expressed, and inducible NOS (iNOS or NOS2) induced in response to inflammation. The enzyme arginase competes with NOS for arginine, promoting the production of ornithine and urea rather than NO. ADMA competitively inhibits NOS, whereas symmetric dimethylarginine enhances oxidative stress and, at high concentrations, impairs arginine uptake by cells, directly and indirectly preventing arginine conversion to NO (37).

The arginine–NO pathway is an important regulator of vascular development, and altered arginine and NO bioavailability may impair vasculogenesis and angiogenesis and negatively affect fetal growth and survival (38). NO is a gaseous signaling molecule initially identified for its vasodilatory activity, acting via soluble guanylate cyclase (sGC) in smooth muscle cells to control vascular tone. It has since been shown to have critical roles in regulation of neurotransmission, angiogenesis, inflammation, and cell adhesion by mechanisms including activation of sGC and by s-nitrosylation of numerous regulatory proteins (39, 40). At the endothelial interface, NO regulates critical mediators of angiogenesis including the vascular endothelial growth factor (VEGF) [e.g., placental growth factor (PlGF) and its receptor soluble fms-like tyrosine kinase-1 (sFlt1)] and angiopoietin-tunica interna endothelial cell kinase 2 (Tie-2) (e.g., angiopoietin-1 and angiopoietin-2 and their receptor soluble Tie-2) protein families. The induction of angiogenesis by angiopoietin and VEGF signaling is dependent on protein kinase–mediated downstream activation of eNOS in the endothelium (and in pregnancy, placental cytotrophoblasts and syncytiotrophoblasts), and consequent production of NO from arginine (30, 38, 41, 42). Collectively the available evidence implicates dysregulation of arginine–NO biogenesis in a range of vascular pathologies including cardiovascular disease and pre-eclampsia (43, 44).

L-citrulline is produced as a by-product of NO synthesis from arginine and can be recycled in a feedback loop to
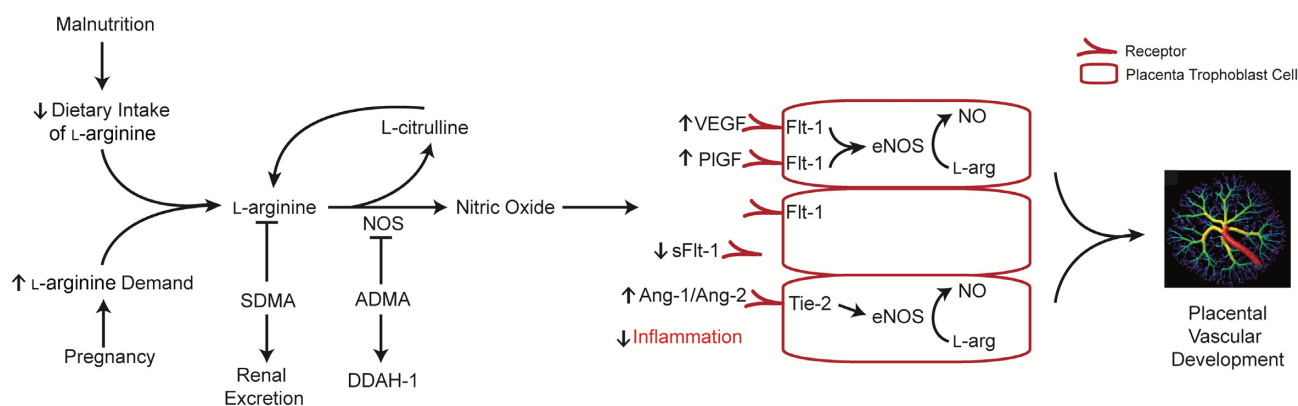

FIGURE 1 The L-arginine–NO biosynthetic pathway regulates key vasculogenic and angiogenic factors in pregnancy. Demand for L-arginine is increased during pregnancy to support the rapidly growing placenta and fetus. L-arginine is acquired in protein-rich foods, which are often lacking in diets in low-resource settings. Therefore, reduced dietary intake, in combination with increased demand in pregnancy, may place pregnant women at risk of arginine deficiency and reduced bioavailable NO. L-arginine is converted to NO via NOS enzymes. L-citrulline, which is also acquired through dietary intake, is produced as a by-product of NO synthesis from L-arginine, and can be recycled in a feedback loop to generate more arginine. Several enzymes and competitive inhibitors such as arginase, ADMA, and SDMA influence the equilibrium of arginine use between metabolic pathways and affect the bioavailability of NO. ADMA competitively inhibits NOS, whereas SDMA can inhibit the cellular uptake of L-arginine, directly and indirectly preventing L-arginine conversion to NO. The enzyme DDAH degrades methylarginines, including ADMA. At the placental trophoblast, NO interacts with critical mediators of angiogenesis (PlGF, VEGF, the angiopoietins). The induction of angiogenesis by angiopoietin and VEGF proteins is dependent on downstream activation of eNOS and production of NO from L-arginine. NO signaling also acts in a positive feedback loop to increase endothelial amounts of VEGF and Ang-1. Furthermore, NO reduces inflammation-mediated endothelial activation by reducing expression of vascular adhesion molecules and proinflammatory cytokines. ADMA, asymmetric dimethylarginine; Ang-1/-2, angiopoietin-1 and -2; DDAH-1, dimethylarginine dimethylaminohydrolase-1; eNOS, endothelial nitric oxide synthase; NOS, nitric oxide synthase; PlGF, placental growth factor; SDMA, symmetric dimethylarginine; sFlt-1, soluble fms-like tyrosine kinase-1; Tie-2, tunica interna endothelial cell kinase 2; VEGF, vascular endothelial growth factor.

generate more arginine. Citrulline is also acquired via dietary intake and through synthesis from glutamine, glutamate, and proline in the intestine (35). The conversion of citrulline to arginine in the intestinal–renal axis accounts for most de novo arginine production (35, 45). Disease states with reduced L-citrulline production (i.e., sepsis) exhibit decreased bioavailable arginine and NO, contributing to pathology (46). During conditions of decreased dietary arginine intake, as in many resource-constrained settings (where L-citrulline intake would also be low), de novo endogenous synthesis of arginine from L-citrulline is not enhanced to compensate (47). Therefore, bioavailable arginine necessary for optimal physiologic function may be outstripped in conditions of simultaneously increased metabolic demand (e.g., pregnancy, infection) and decreased dietary arginine and citrulline.

In addition to NO, several other arginine metabolites including polyamines, creatine, and proline are also necessary for healthy pregnancies (48, 49). Creatine is required for fetal neurological development and muscle function, whereas proline is an important amino acid for cartilage development (48, 49). Polyamines interact with RNA and DNA to modulate protein synthesis and cell growth and function (50). Polyamines are synthesized from L-arginine via the agmatine and ornithine metabolic pathways and are required through several stages of pregnancy, including implantation, early embryogenesis, fetal growth, and placental development (51, 52). Pharmacological and diet-driven reductions in polyamine bioavailability in pregnant rodents and large mammals have been associated with abnormal placentation, FGR, and pregnancy loss (51, 52).

NO also plays several regulatory roles throughout placental development, beginning with its role as a facilitator of implantation and endovascular trophoblast invasion (30, 38, 53, 54). The processes of trophoblast cell migration and invasion, vessel development, and vessel maturation in the placenta are facilitated by the VEGF and angiopoietin pathways, which induce NO production in the endothelium (26, 30, 38, 55, 56). Early in pregnancy, eNOS is also expressed in several placental cell types including cytotrophoblasts, extravillous trophoblasts, and syncytiotrophoblasts (38). As pregnancy progresses, placental eNOS expression increases and becomes localized primarily to syncytiotrophoblasts and vascular endothelial cells (42, 57). In the absence of eNOS, VEGF does not exhibit its regular angiogenic effects (58). NO also promotes a proangiogenic environment by increasing trophoblast expression of PlGF and VEGF and decreasing expression of the antiangiogenic factor sFlt1 (59). Recent evidence has implicated changes in angiogenic factors mediated by NO signaling (e.g., PlGF, VEGF, sFlt1, angiopoietin-1/2), as well as NO itself, in placental insufficiency and adverse pregnancy outcomes including gestational hypertension, pre-eclampsia, PTB, and FGR (34, 60–69). Factors that decrease the ability of eNOS to convert arginine to NO (i.e., increased ADMA or decreased bioavailable arginine) have also been implicated in the pathobiology of pregnancy disorders including FGR and pre-eclampsia (70–73). After

placental vasculature is established, NO acts as a vasodilator by activating sGC in vascular smooth muscle cells to induce relaxation. NO-mediated vasodilation is necessary during pregnancy to modulate maternal blood pressure, systemic blood flow, and adequate placental perfusion to support fetal growth. NO synthesis is required to decrease the risk of pregnancy-related hypertensive disorders, including pre-eclampsia and eclampsia.

Collectively these data indicate there is an increased demand for bioavailable NO, polyamines, and arginine during pregnancy in order to support placental and fetal growth and function. In humans, there is a physiological increase in arginine synthesis during the second trimester, a decrease in circulating ADMA, and a corresponding increase in NO synthesis (74, 75). Despite increased in vivo production, however, pregnancy has been described as a state of arginine deficiency because endogenous production cannot meet the increased demand from placental and fetal growth (76). Thus, exogenous intake of arginine is particularly important for pregnant women and dietary supplementation may be required in situations where dietary intake is inadequate.

Arginine Nutritional Requirements, Dietary Sources, and Supplementation in Pregnancy

Protein-rich foods such as meats, dairy products, nuts, seeds, soy, and pulses are considered to be rich in arginine. The relative amount of arginine in various proteins ranges from 3% to 15% (77, 78). Most cereals are deficient in arginine, with only 3–4% of their low protein content derived from arginine (78). Vegetables and fruits contain a negligible amount of protein and thus contain little arginine. As a result, variation in dietary patterns could account for the differences in plasma arginine observed globally (78). In resource-constrained settings, like sub-Saharan Africa, only 9% of dietary energy comes from arginine-rich foods such as meats, pulses, and seeds, whereas >60% comes from arginine-poor roots, tubers, and cereal-based staples (12). Therefore, patterns of dietary intake indicate the potential for widespread arginine deficiency in these areas, especially in pregnant women. Arginine is now considered to be nutritionally essential for optimal health, fertility (i.e., spermatogenesis in human males), and reproduction (79). However, because arginine has traditionally been considered a non- or conditionally essential amino acid in the context of maintaining nitrogen balance (79), comprehensive reference materials for dietary arginine requirements have not been compiled.

Normal dietary intake in Western countries is ∼4–5 g/d (36). The tolerable upper intake limit of arginine supplementation, especially over long periods, has not been definitively set (80). Studies in pigs and rats have not reported adverse effects with supplemental doses ≤630 mg arginine · kg body weight^{-1} · d^{-1} and 3.6 g arginine · kg body weight^{-1} · d^{-1}, respectively, for 91 d (81). Using conversion factors provided by the FDA, this translates to an estimated

40 g/d for a 70-kg human adult (81, 82). Human trials of arginine supplementation in adults have been inconsistent in dose, duration, and sample population; however, the absence of reported adverse events remains consistent (81, 83). The observed safe amount for arginine supplementation in adults is currently 30 g/d for 90 d (84). Mild gastrointestinal side effects have been reported after large amounts of arginine in a single dose, but using multiple smaller doses is reported to reduce gastrointestinal intolerance (84).

The required dose of arginine during pregnancy has not been determined. However, the daily average intake of arginine is 4.3 g/d among pregnant women in the United States, a country where arginine intake is considered to be sufficient (85). Arginine consumption has been estimated at ≤2–3 g/d in low-resource settings, well below the intake of women in resource-rich settings (12). Pregnancy is a state of relative arginine deficiency and infections such as malaria further deplete arginine (86); therefore, the low dietary intake of arginine in resource-constrained, malaria-endemic settings may put pregnant women at risk of hypoargininemia. The critical role that the arginine–NO biosynthetic pathway plays in regulating vascular development and other pathways important for healthy pregnancy outcomes suggests that supplemental arginine in LMICs may reduce adverse birth outcomes.

A supplement-based strategy is generally identified as the most effective approach for populations that have a high prevalence of nutrient deficiency and for target groups where a required nutrient is difficult to attain through normal diet (e.g., LMICs) (87). Possible supplementation platforms for arginine include syrups, food bars, tablets (chewable or effervescent), caplets, and powders (including crystalline forms of arginine like arginine-HCl and arginine α-ketoglutarate). Supplementation of arginine and L-citrulline in ready-to-use supplementary food in Tanzanian children with sickle-cell disease reported no issues with tolerability and a compliance rate of 95% (88), suggesting this may be an effective formulation for arginine supplementation in LMICs.

One challenge in arginine supplementation is arginase, which catalyzes the metabolism of arginine into urea and is highly active in the intestine of adults. Only 60% of oral arginine evades intestinal metabolism (47) and another 15% of circulating arginine is metabolized in the liver (89). An approach to circumvent arginine metabolism is the administration of L-citrulline, which can be converted into arginine but is not a substrate for arginase (90). Thus, coadministration of L-citrulline and arginine could bypass this barrier and increase the efficacy of arginine supplementation. Recent studies support the effectiveness of oral L-citrulline in increasing bioavailable arginine concentrations and NO-dependent signaling (91, 92). The combination of oral L-citrulline and arginine supplementation in adult males increased plasma arginine more effectively than did either supplement alone (93). These studies support the hypothesis that supplementation with arginine plus L-citrulline would be the most effective strategy to increase bioavailable arginine and NO and improve birth outcomes.

Studies of Arginine Supplementation in Pregnancy

A large body of work has investigated arginine supplementation in pregnancy in mammals (49, 94). Supplementation with arginine and other members of arginine metabolic pathways (e.g., glutamine) results in increased placental growth (95, 96), fetal viability (95, 97–99), litter size (95, 96, 100), and birth weight (49, 95–100) in pigs and sheep. Animal models using low-protein diet–induced FGR have reported increases in NO production, fetal:placental weight ratio, and fetal birth weight in animals receiving arginine and L-citrulline supplementation (101, 102). These preclinical data demonstrate the potential of arginine supplementation to improve birth outcomes in the context of low-protein diets observed in pregnant women in LMICs. Arginine supplementation in human pregnancy has not been evaluated in low-income settings; however, studies from high- and middle-income countries have reported benefits of arginine supplementation in pre-eclampsia, gestational hypertension, FGR, and preterm labor (**Table 1**).

Arginine supplementation in pre-eclampsia

Pre-eclampsia, similar to malaria in pregnancy, is characterized by endothelial dysfunction and placental insufficiency, and there is evidence for dysregulation of the arginine–NO pathway in its pathophysiology (43, 71, 73). An initial trial of arginine supplementation in pre-eclampsia was underpowered and low quality, although it reported reductions in maternal blood pressure (103). Better-designed, yet still generally underpowered randomized controlled trials (RCTs) followed. Two evaluated longer-term, low-dose arginine supplementation (3 g/d for 3 wk) in pre-eclampsia and observed significant improvements to maternal and fetal hemodynamics, as well as better fetal outcomes (107, 108). In contrast, 2 RCTs in pre-eclamptic women using short-term, moderate doses initiated later in pregnancy (e.g., 12 g/d for 5 d) observed no benefit (104, 105). These data suggest that short-term supplementation, especially later in pregnancy, is insufficient to improve maternal hemodynamics.

The aforementioned observations are consistent with human and preclinical data indicating that interventions to affect placental vascular insufficiency via the arginine–NO pathway need to be initiated early and continued over the course of pregnancy (118–120). In support of this hypothesis, the best-designed and largest RCTs to date examined low-dose long-term arginine supplementation in pre-eclampsia (3 g or 6.6 g/d), initiated early in pregnancy (~20 weeks of gestation) (76, 106). These studies reported arginine supplementation was associated with persistent decreases in maternal blood pressure and resulted in significantly improved fetal and maternal outcomes in pregnancies at risk of pre-eclampsia (76, 106).

TABLE 1 Characteristics of clinical studies providing L-arginine during pregnancy[1]

Study (ref.)	Country	Dose (g/d)	Delivery form	Duration	Group	Treatment groups (n)	Key findings
PE studies							
Facchinetti et al. (103)	Italy	30	i.v.	Single dose	n = 29	PE: uncomplicated pregnancy (12), PE (17)	• L-arginine–induced reduction in SBP and DBP in both groups, greater decrease of DBP in women with PE • Increased serum L-citrulline during L-arginine treatment • Total L-citrulline production inversely related to baseline blood pressure, but not associated with birth outcomes
Hladunewich et al. (104)	United States	14 (oral) or 30 (i.v.)	Oral (i.v. if could not take oral)	Varied by participant: ≥3 d postpartum (some began predelivery)	n = 67 (n = 45 received treatment)	PE: L-arginine (22) or placebo (23), and healthy gravid controls (22)	• Increased baseline ADMA, cGMP, and endothelin in PE compared with healthy controls • Increased postpartum serum L-arginine in the L-arginine treatment group • No changes in postpartum serum NO, endothelin-1, cGMP, ADMA • No change in postpartum glomerular filtration rate, blood pressure, proteinuria
Staff et al. (105)	Norway	12	Oral	≤5 d	n = 30	PE: L-arginine (15) or placebo (15)	• No change in DBP at 2 d post-intervention • No difference in latency to delivery, or mean birth weight
Vadillo-Ortega et al. (76)	Mexico	6.6	Oral	Duration of pregnancy after enrollment (14–32 weeks of gestation)	n = 672	PE: women with previous PE or high risk; placebo (222), L-arginine + antioxidant vitamins (228), antioxidants alone (222)	• Plasma L-arginine (before treatment) lower in women who later developed PE • Reduced incidence of PE in L-arginine–treated group compared with placebo • Benefit of L-arginine and antioxidant treatment over treatment with antioxidant vitamins alone • Increased circulating L-arginine and decreased SBP and DBP in L-arginine + vitamins group • Reduced risk of PTB in women who received L-arginine
Camarena Pulido et al. (106)	Mexico	3	Oral (capsules)	Duration of pregnancy after enrollment (20 weeks of gestation)	n = 96	High risk of PE: L-arginine treated (49) or placebo (47)	• Increased incidence of PE, specifically severe PE, in placebo-treated women • Increased birth weight and decreased PTB in L-arginine–treated group • Decreased SBP and DBP and mean arterial pressure
Rytlewski et al. (107)	Poland	3	Oral	3 wk	n = 61	PE: L-arginine (30) or placebo (31)	• Lower SBP, DBP, and mean arterial blood pressure in L-arginine–treated group • L-arginine increased 24-h urinary excretion of NOx and mean plasma L-citrulline • No change in plasma L-arginine, L-ornithine, ADMA, SDMA, or L-NMMA
Rytlewski et al. (108)	Poland	3	Oral	Duration of pregnancy after enrollment (average enrollment at 29 weeks of gestation)	n = 61	PE: L-arginine (30) or placebo (31)	• L-arginine reduced pulsatility indexes in umbilical artery and increased pulsatility indexes in the middle cerebral artery, as well as increased cerebro-placental ratio values (MCA/UA) • Decreased antihypertensive dosage in patients receiving L-arginine • Lower rates of FGR, increased latency to delivery, and higher Apgar scores in L-arginine group

(Continued)

TABLE 1 *(Continued)*

Study (ref.)	Country	Dose (g/d)	Delivery form	Duration	Group	Treatment groups (n)	Key findings
Gestational hypertension studies							
Neri et al. (109)	Italy	20	i.v.	Single dose	n = 15	Mild to moderate gestational hypertension	• No impact on fetal movements or fetal heart rate • Acute arginine treatment reduced maternal SBP and DBP
Neri et al. (110)	Italy	20	i.v.	5 d	n = 123	Gestational hypertension: L-arginine (62) or placebo (61)	• Reduced SBP and DBP by both placebo and L-arginine, with greater decrease in L-arginine group • Improved performance of L-arginine (impact on blood pressure) in a subgroup analysis of women not receiving antihypertensives
Facchinetti et al. (111)	Italy	20 (i.v.), 4 (oral)	i.v. + oral	5 d i.v., then 2 wk oral	n = 74	Gestational hypertension with or without proteinuria: placebo (35) or L-arginine (39)	• Increased latency (to delivery) in L-arginine–treated group • Reduced SBP and DBP 6 d post-i.v. treatment • L-arginine–associated trend towards increased latency, attenuated evolution of PE, and reduced low birth weight in subset of patients without proteinuria
Neri et al. (112)	Italy	4	Oral	Duration of pregnancy after enrollment (<16 weeks of gestation)	n = 79	Mild chronic hypertension: L-arginine (39) or placebo (40)	• No difference in SBP or DBP, but fewer women in the L-arginine–treated group were given antihypertensive medications • Increased gestational age, birth weight, and trend towards reduced neonatal complications in L-arginine group
Adverse birth outcome studies							
Neri et al. (113)	Italy	30	i.v.	Single dose	n = 27	FGR: AGA (9), and FGR with (9) or without (9) increased utero-placental resistance	• No hemodynamic changes in utero-umbilical circulation • L-arginine–induced decrease in resistance (nonplacental side) in FGR pregnancies with increased baseline resistance • Increased plasma NOx and growth hormone concentrations after L-arginine treatment in all groups
Winer et al. (114)	France	14	Oral	Duration of pregnancy after enrollment (24–32 weeks of gestation)	n = 43	Severe FGR: L-arginine (21) and placebo (22)	• No change in birth weight or maternal or neonatal characteristics • No impact on outcomes in pregnancies with severe vascular growth restriction • No difference in serum NOx
Sieroszewski et al. (115)	Poland	3	Oral	20 d	n = 108	FGR: L-arginine (78) or no treatment (30)	• Increased fetal weight gain (during treatment) in the L-arginine–treated group • Increased newborn weight
Singh et al. (116)	India	3	Oral	21 d	n = 120	FGR: women with FGR (60; 30 received L-arginine, 30 placebo) or AGA fetal growth (60)	• Before treatment, lower serum NO in FGR than in AGA • Increased serum NO in L-arginine–treated FGR • Reduced umbilical artery resistance in L-arginine–treated FGR group • L-arginine increased live births, birth weight, and reduced neonatal complications (nonsignificant)
Rytlewski et al. (117)	Poland	3	Oral	Varied by participant (average of 3 wk)	n = 45	Preterm labor: L-arginine (25) or placebo (20)	• L-arginine reduced pulsatility indexes in umbilical artery and increased pulsatility indexes in the middle cerebral artery, as well as increased cerebro-placental ratio values (MCA/UA) • No change in markers from NO pathway (plasma L-arginine, L-citrulline, L-ornithine, NOx) • No change in pregnancy outcomes

[1] Only original human studies of arginine supplementation in pregnancy are included. Animal studies and systematic reviews/meta-analyses are not included. ADMA, asymmetric dimethylarginine; AGA, appropriate for gestational age; cGMP, cyclic guanosine monophosphate; DBP, diastolic blood pressure; FGR, fetal growth restriction; L-NMMA, L-N^G-monomethyl arginine; MCA/UA, middle cerebral artery/umbilical artery; NOx, nitrate/nitrite; PE, pre-eclampsia; PTB, preterm birth; SBP, systolic blood pressure; SDMA, symmetric dimethylarginine.

Arginine supplementation in gestational hypertension

Four published RCTs have investigated arginine supplementation in the context of gestational hypertension (Table 1). The first 2 studies assessed the acute effects of large doses (20 g intravenously once, or for 5 d) relatively late in pregnancy (109, 110). Whereas 1 study was small ($n = 15$) (109), the other was a larger ($n = 123$) multicenter RCT (110). Both studies reported that parenteral arginine decreased maternal blood pressure but neither reported on pregnancy outcomes. In the absence of evidence of improved clinical outcomes, and given the challenges of long-term parenteral arginine administration, it is unclear whether this is a feasible therapeutic approach. In contrast, 2 subsequent RCTs ($\sim n = 70$) using longer-term, low-dose oral arginine (4 g/d) reported reduced maternal blood pressure (and/or reduced antihypertensive use) and improved pregnancy outcomes (111, 112).

Two meta-analyses of studies of arginine supplementation in the context of gestational hypertension and pre-eclampsia reported significantly decreased maternal diastolic blood pressure and extended length of pregnancy (121, 122). Collectively, the existing data support the use of longer-term low-dose arginine supplementation as the regimen most likely to improve maternal and fetal outcomes in hypertensive disorders of pregnancy.

Arginine supplementation and adverse birth outcomes

Many of the trials summarized in Table 1 evaluated adverse birth outcomes as either a primary or a secondary outcome (Table 1). Two trials that gave short-term arginine supplementation to women with pre-eclampsia reported no difference in birth outcomes between the 2 trial arms (104, 105). As above, these data are consistent with the hypothesis that short-term arginine supplementation initiated later in pregnancy is insufficient to reverse underlying vascular pathobiology. In contrast, the RCTs that used low-dose arginine supplementation early and continued it across pregnancy reported increased birth weight, length of gestation, and/or reduced rates of PTB (76, 106, 108, 111, 112).

Additional studies investigated arginine supplementation in the context of pregnancies complicated by FGR and preterm labor (Table 1). An initial report of arginine to treat FGR used a single arginine infusion (30 g intravenously) and reported improvements to utero-placental circulation (113). However, this trial was underpowered ($n = 27$), uncontrolled, and relied on subgroup analyses, limiting its conclusions (113). Two larger RCTs of low-dose, long-term arginine supplementation (3 g/d for 3 wk) in pregnancies complicated by FGR reported improved neonatal outcomes and significantly increased fetal weight gain (115, 116).

Two trials of arginine supplementation in pregnancies with threatened adverse birth outcomes reported no benefit. One well-designed but small ($n = 43$) multicenter RCT reported no difference in outcome in women with severe vascular FGR receiving either arginine (14 g/d) or placebo (114). In another small trial of women presenting in preterm labor ($n = 45$), low-dose arginine supplementation (3 g/d) improved maternal and fetal hemodynamics but did not significantly improve birth outcomes (117). Both studies tested prolonged treatment with low dosage (114, 117); however, they enrolled severe cases of FGR or threatened PTB. Together these data indicate that arginine supplementation alone may not be sufficient to reverse advanced pathology including existing preterm labor or severe vascular FGR.

Summary and future directions for arginine supplementation studies

In summary, trials that reported significantly improved birth outcomes in women who received arginine compared with placebo (76, 106, 108, 111, 112, 115) used a prolonged, oral low-dose (3–7 g/d) regimen. Whereas, placebo-controlled trials that reported no difference in birth outcomes were in cases of advanced pathology (i.e., severe vascular FGR or preterm labor) (114, 117) or short-term treatment strategies initiated later in pregnancy (104, 105). To date, the largest ($n = 672$) well-designed RCT provided the strongest evidence that early initiation of low-dose arginine supplementation may improve pregnancy outcomes (76). In this study, women who began oral arginine supplementation (6.6 g/d) before 24 weeks of gestation had reduced rates of pre-eclampsia compared with placebo controls. Biologically, early and prolonged arginine dosing over pregnancy aligns with the important role of the arginine–NO biosynthetic pathway in regulating the critical processes of angiogenesis and vasculogenesis required for normal placental vascular development and healthy pregnancy outcomes. Once disrupted, these developmental pathways are unlikely to be reversed with late or single-dose approaches. Collectively the published human data support the hypothesis that supplementation with a daily physiological dose of arginine mimicking regular dietary intake (rather than a supraphysiological one-time dose) is beneficial to pregnancy outcomes in at-risk pregnancies.

Although mild gastrointestinal upsets such as dyspepsia were reported in large arginine supplementation trials in pregnancy (76, 106), these adverse events did not lead to participants discontinuing involvement in the studies. No study to date has reported any severe adverse events attributed to arginine supplementation in pregnancy. A limitation of the existing body of evidence is that most studies, with the exception of one (76), drew conclusions from relatively small sample sizes. Additional large RCTs, powered to investigate the impact of arginine on birth outcomes including FGR and PTB in at-risk pregnancies such as those in LMICs, are needed. Despite evidence that coadministration of citrulline and arginine may be a more effective strategy to increase bioavailable arginine and improve pregnancy outcomes in preclinical models (101, 102), no published human studies to date have examined the impact of supplementation with citrulline or a combination of arginine and citrulline on birth outcomes.

Dietary Arginine Intake and Impact on Birth Outcomes in Resource-Constrained Settings

Most research on arginine supplementation has been conducted in high-resource settings. The burdens of adverse birth outcomes, maternal malnutrition, and infections (i.e., malaria) that deplete arginine are greatest in LMICs, and therefore the benefit of arginine supplementation would be expected to be the largest in LMICs. There is preliminary evidence for an association between arginine and adverse birth outcomes in LMICs, but no existing studies have directly assessed arginine supplementation in LMICs. A recent study of >7000 Tanzanian pregnant women reported reduced risk of PTB in women with higher dietary arginine intake (123). Pan-African population-based analyses indicate dietary arginine deficiencies in resource-constrained settings (12) and therefore a greater risk of lower NO bioavailability during placental development. However, there is a need for RCTs in LMICs to address the hypothesis that arginine supplementation in pregnancy would improve birth outcomes in these settings.

Many LMICs (especially in sub-Saharan Africa, South Asia, and Southeast Asia) are endemic for malaria. In these regions malaria in pregnancy is a major contributor to poor birth outcomes, and a growing body of evidence is documenting the impact of malaria infection on bioavailable arginine and NO. Reduced circulating arginine concentrations and NO bioavailability, and a lower ratio of arginine to ADMA (a measure of arginine bioavailability) are well described in the context of malaria infection and correlate with endothelial dysfunction and disease severity (124–132). In these studies, recovery from severe malaria was associated with increases in circulating arginine (127), and intravenous arginine treatment of patients with severe malaria improved endothelial function and increased exhaled NO (126). Malaria-associated reductions in arginine and NO bioavailability may be attributed to increased circulating arginase and NO-scavenging cell-free hemoglobin released during malaria-induced hemolysis (86).

A study of Malawian women showed that malaria infection during pregnancy increased ADMA and symmetric dimethylarginine, and decreased bioavailable arginine (118). Malaria infection in early pregnancy was associated with reduced arginine concentrations and higher ADMA concentrations across pregnancy, and elevated ADMA early in pregnancy increased the relative risk of a small-for-gestational-age delivery. Furthermore, measures of better maternal nutrition (e.g., higher midupper arm circumference and hemoglobin) were associated with increased circulating arginine, decreased ADMA, and increased ratio of arginine to ADMA. Although no human trials have yet reported arginine supplementation in the context of malaria in pregnancy, arginine supplementation in a preclinical mouse model of malaria in pregnancy enhanced placental vascular development and improved fetal weight and viability (118). These studies suggest that in settings with high rates of maternal malnutrition and malaria infection, maternal hypoarginemia could be a preventable and modifiable risk factor contributing to adverse birth outcomes such as PTB and FGR.

Conclusion

The burden of adverse birth outcomes in resource-constrained settings is disproportionately high (1). New interventions appropriate for implementation in LMICs are needed to reduce poor birth outcomes and enable all children to survive and thrive. Population-level data of dietary arginine intake suggest that many women in LMICs are not receiving adequate dietary arginine required for successful pregnancy outcomes. Many LMICs are also malaria-endemic, an infection that has been shown to further deplete circulating arginine in pregnancy and contribute to placental insufficiency and adverse birth outcomes. Arginine deficiency in LMICs represents a modifiable risk factor to promote healthy birth outcomes. Based on the characteristics of food consumption in LMICs (e.g., food insecurity, growing food at home) and the large amount of daily arginine required in pregnancy (4–5 g/d; possibly more in malaria-endemic settings), we hypothesize that supplementation would represent an effective intervention strategy. We further propose that a combination supplement containing arginine and L-citrulline may most efficiently increase bioavailable arginine and NO in pregnant women.

Considering the preclinical and clinical evidence presented in this Perspective, we propose that a multisite RCT that evaluates ∼7 g/d each of supplemental arginine and citrulline compared with placebo given to pregnant women in malaria-endemic areas, beginning early in pregnancy (<20 weeks of gestation) and continued over the course of pregnancy, would be an important next step to evaluate the potential of arginine to improve birth outcomes in LMICs.

The majority of preterm deliveries and adverse birth outcomes, including FGR, occur where women do not have access to adequate nutritional support. Preventing adverse birth outcomes (e.g., PTB, FGR) is one of the most cost-effective strategies to improve global health and promote healthy life trajectories for young children. Confirming the safety, efficacy, cost-effectiveness, and scalability of arginine supplementation during pregnancy in reducing adverse birth outcomes would represent a major advance towards an affordable, culturally acceptable, and potentially life-saving intervention suitable for implementation and scale-up in low-resource settings.

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